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14. ABSTRACT

The hypothesis that forms the basis for this research is that patients who possess certain SNPs or CNPs are at a greater risk for developing severe urinary morbidity or ED resulting from radiotherapy for prostate cancer. The specific aim of this project is to identify through a genome wide association study the SNPs and CNPs associated with the development of severe urinary morbidity and ED resulting from the use of radiation to treat prostate cancer. It should be noted that we may also identify SNPs or CNPs that are associated with protection against the development of these forms of radiation injury. The main accomplishment of the third year was substantial progress on the validation phase of the project. Specifically, we selected approximately 5,000 SNPs from the discovery phase of the GWAS and have begun to genotype the 600 samples that comprise the validation cohort.

15. SUBJECT TERMS
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INTRODUCTION:

Radiotherapy can provide a sustainable cure for prostate cancer and has become accepted as a standard treatment option. However, some men develop side effects following treatment, including urinary morbidity and erectile dysfunction, which have a substantial effect on quality of life. These side effects vary in duration and severity, and while most patients return to baseline symptom levels after a year, a subset of patients experience more severe and lasting effects. A predictive assay that could identify such patients could be used to help tailor treatments plans. Previous research on radiation induced injury in breast cancer patients suggests that the variation in such side effects is largely due to patient-specific, possibly genetic effects rather than treatment differences or random effects. The purpose of the current study is to identify genetic polymorphisms associated with development of either urinary morbidity or erectile dysfunction following radiotherapy for prostate cancer. The medical application of these findings will be to develop a risk assessment genetic test to assist physicians and patients in making informed decisions on the course of therapy for prostate cancer. Physicians and patients could together weigh the benefits of therapy with the individualized risk of developing radiation side effects and could then customize the treatment course.

BODY:

Efforts in the third year of funding have been focused on the validation phase of the genome-wide association study. From the Discovery phase, completed during the second year of funding, we identified approximately 5,000 SNPs and CNP markers associated with one or more radiation-induced adverse effects under investigation. We then designed a custom SNP array that was created by Illumina (San Diego, CA), and we are currently completing genotyping among 595 patients comprising the validation cohort.

Advances in technology that took place during the second year of the project, have allowed us to increase both our SNP selection limit and sample size for the validation study. For similar cost to doing TaqMan assays as planned, we were able to build a custom microarray using Illumina's Infinium iSelect HD custom genotyping platform to genotype samples in the validation cohort. This allowed us to select approximately 1% of the SNPs from the discovery cohort for validation rather than the more modest numbers that would have been feasible using the TaqMan assay. Furthermore, for the same cost, we were also able to increase our sample size for the validation cohort from ~300 to 595 patients. Table 1 describes the patients selected for inclusion in the validation study.

Because the custom array allows us to select a higher proportion of SNPs, we were able relax our otherwise conservative SNP selection criteria. Initially, we had set the type I error rate at 0.0001, allowing us to detect SNPs and CNPs with effect size of ~2.5 or greater. This would have resulted in selection of approximately 0.1% of the SNPs for genotyping in the validation study. However, since the custom microarray allowed us to type approximately 1% of the SNPs investigated in the discovery study, we were able to lower our type I error rate to 0.001, thereby allowing us to include SNPs that would have been otherwise thrown out as false negatives using the more stringent type I error threshold. We recognize that we are also increasing the number of false positives that will be carried over into the validation study, but we are confident we will be able to distinguish the true positives using a joint analytic approach whereby the p-values from the discovery and validation phases are combined to increase power [1].

In our proposal, in addition to selecting SNPs found to be significantly associated with radiation adverse effects in the discovery phase, we planned to include SNPs that are likely to affect genes functionally involved in radiation response. To this end, we have worked with collaborators from Washington University School of Medicine in St. Louis and collaborators from the University of Cambridge in the UK to select such candidate SNPs. Specifically, we included in the validation study 104 SNPs that lie in genes that have been shown in published studies to play a role in radiation response pathways such as DNA damage repair, inflammation, and apoptosis. We also included 95 SNPs that were identified recently in the discovery phase of a similar GWAS currently underway and shared with us by our collaborators at the University of Cambridge.

Because this study involves a multi-ethnic patient population, and ancestry was adjusted for in the analysis of the discovery phase data, we have selected approximately 1,000 ancestry-informative markers for inclusion on the custom array being used in the validation study. To do this, we performed principle components analysis using reference populations from three sources: the International HapMap Project, the Population Reference Sample (POPRES), and the Human Genome Diversity Project (HGDP)[2-4]. We selected SNPs with minor allele frequency differences between pairs of reference populations, and then, using principle components analysis, tested the ability of various sized panels of selected 'ancestry-informative' SNPs to distinguish the ethnically and geographically distinct reference populations. We compared the performance of our ancestry-informative SNPs to a random selection of 100,000 SNPs which is typically used for principle components analysis. We found that we could adequately stratify population groups using approximately 950 SNPs. These SNPs were included on the custom array and will be used in the validation study to calculate principle components for ancestry-adjustment in regression models.

We began building the custom SNP arrays in June, and, using the services of the Institute for Genomics and Multiscale Biology at Mount Sinai School of Medicine, are completing genotyping all 595 patients in the validation cohort. We have been granted a no-cost extension of one year, and will spend this time on final analysis of the data from the validation study and manuscript preparation. In preparation for this, we have finalized our analyses of clinical predictors of radiation adverse effects that will be included in the SNP analysis. Patient-related variables include age, pre-treatment symptoms (urinary symptoms and erectile function), use of hormone therapy, hypertension, diabetes, and smoking status. Treatment-related variables include total biologically effective dose, prostate D90 (minimum dose to 90% of the prostate volume), and whether the patient received external beam RT in addition to brachytherapy. We are currently in the process of completing QC checks on the validation cohort data, and will then begin statistical analysis using multivariate regression models to investigate each SNP as well as combinations of significant SNPs.

KEY RESEARCH ACCOMPLISHMENTS:

- Designed and built a mid-plex custom SNP microarray to genotype approximately 5,000 SNPs identified in the discovery phase of the project as well as approximately 1,000 ancestry-informative markers
- Worked with collaborators in the UK and US to select approximately 200 additional candidate SNPs on the basis of functional involvement in radiation response
- Genotyped approximately 600 patients comprising the validation cohort for discovery phase SNPs and candidate SNPs
- Developed regression models incorporating clinical and covariate that will be used to analyze each SNP in the validation study

Reportable Outcomes

While our validation phase samples were being processed and genotyped, we utilized the GWAS data from the discovery phase of the project to investigate a number of candidate gene SNPs and SNPs identified by our collaborators who are also carrying out radiotherapy response GWAS. We were able to identify 8 SNPs in 6 genes that are significantly associated with late urinary morbidity following radiation therapy (Table 2). Specifically, we identified rs11571468 and rs11571435 in RAD52 (corrected p-value 1.49E-04 and 1.29E-04), rs9350 and rs4150005 in EXO1 (corrected p-value 2.98E-03 and 2.75E-03), rs1554132 in TP63 (corrected p-value3.67E-03), rs2345060 in PMS2 (corrected p-value 0.022), rs2972357 in CDK7 (corrected p-value 0.054), and rs817000 in LIG4 (corrected p-value 0.034). We have shared out findings with our collaborators in the UK, and they are in the process of determining if these SNPs replicate in their cohort of prostate cancer radiotherapy patients.

Conclusions

Our results to date support the feasibility of a genome-wide association study to identify genetic variants associated with radiotherapy adverse response. The results of this study should provide the basis for development of a clinically relevant predictive test to identify patients at increased risk for development of adverse events following radiotherapy. Such a tool could be used to aid clinicians in personalizing dosage to improve the therapeutic index of radiotherapy treatment for prostate cancer.

Appendices

Table 1.

	Discovery Cohort N = 367	***Validation Cohort N = 595***
Age (yrs), mean(sd)	64 (7.3)	66 (7.5)
Stage, n(%)		
T1	200 (54.5%)	293 (49.8%)
T2	154 (42.0%)	271 (46.1%)
T3	13 (3.5%)	22 (3.7%)
Gleason, n(%)		
≤ 6	293 (65.1%)	353 (60.0%)
7	96 (26.2%)	152 (25.9%)
≥ 8	32 (8.7%)	83 (14.1%)
Pre-RT PSA (ng/ml), mean(sd)	9.4 (17.6)	8.6 (7.8)
Prostate CT volume (mm^3), mean(sd)	46.1 (17.6)	47.3 (17.9)
Prostate D90 (Gy), mean(sd)	150.4 (46.5)	149.0 (44.5)
Total BED (Gy), mean(sd)	203.3 (22.5)	199.6 (30.2)
RT type, n(%)		
Brachytherapy	204 (55.6%)	323 (54.9%)
Brachytherapy + EBRT	163 (44.1%)	245 (41.7%)
EBRT	1 (0.3%)	20 (3.4%)
Hormone therapy, n(%)	194 (52.9%)	312 (53.1%)
Smoking status n(%)		
Current	141 (38.4%)	245 (41.7%)
Former	-	-
Never	226 (61.6%)	343 (58.3%)
Diabetes, n(%)	19 (5.2%)	45 (7.7%)
Hypertension, n(%)	131 (35.7%)	182 (31.0%)
Follow-up (months), mean(sd)	47.9 (12.5)	44.2 (14.8)

Table 2.

dbSNPrsID	***Nearest Gene***	***Chr***	***Genotype***	***Mean change in post-RT urinary symptom score***	***Beta (95% CI)***	***p-value***	***Corrected p-value***
rs11571468	RAD52	12p13.33	1/34/196	34.0/5.3/2.8	3.9 (1.6,6.1)	2.76E-06	1.49E-04
rs11571435	RAD52	12p13.33	1/33/196	34.0/5.4/2.8	4.0 (1.,6.3)	2.38E-06	1.29E-04
rs9350	EXO1	1q43	3/31/93	11.7/1.6/0	13.8 (7.2,20.4)	5.51E-05	2.98E-03
rs4150005	EXO1	1q43	3/31/93	11.7/1.6/0	13.8 (7.2,20.4)	5.10E-05	2.75E-03
rs1554132	TP63	3q26	25/76/42	4.9/0.1/0.4	5.0 (2.5,7.5)	6.79E-05	3.67E-03
rs2345060	PMS2	7p22.1	12/66/114	5.6/3.1/0.2	3.1 (1.4,4.9)	4.08E-04	0.022
rs2972357	CDK7	5q13.2	21/78/82	-3.2/2.6/0.8	-4.4 (-7.0,-1.8)	1.00E-03	0.054
rs817000	LIG4	13q33.3	1/47/179	2.0/-0.3/3.2	-3.5 (-5.5,-1.6)	6.20E-04	0.034

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